A STRAIN OF YOSHIDA SARCOMA WITH INDUCED RESISTANCE TO PAFENCYL

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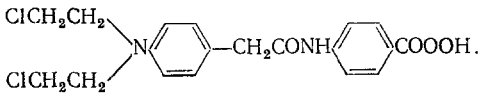
UDC 616-006.3.04-085.277.3:547.581.2]-0.35.2 + 615.277.3:547.2]015.8:616.-006.3.04

KEY WORDS; pafencyl; Yoshida sarcoma; drug resistance.

Pafencyl is the name given to N[p-di(2-chloroethyl)aminophenacetyl]-p-aminobenzoic acid:



This is a new Soviet antileukemic agent, for use in the treatment of chronic lymphatic leukemia, non-Hodgkin lymphomas, lymphogranulomatosis, Waldenstrom's disease, and multiple myeloma [2]. During chemotherapy of malignant tumors resistance develops to the action of active drugs and, in particular, of alkylating agents. To study the phenomenon and the mechanisms of onset of drug resistance to alkylating agents, and also ways of overcoming it, a number of strains of transplantable tumors and leukemias possessing different degrees of resistance have been produced [2, 5].

When pafencyl and other drugs are used clinically, some patients develop resistance to its action [2, 3], and it is therefore important to study this process in an experimental model. The Svec strain of leukemia, resistant to pafencyl, was described previously and has proved to be cross-resistant to all alkylating agents studied [4]. Unfortunately the Svec strain of leukemia is primarily resistant to the action of antimetabolites and alkaloids used in the treatment of leukemias, so that it is impossible to study cross resistance of these groups of drugs on this strain.

The aim of the present investigation was to create a strain of Yoshida sarcoma, resistant to pafencyl, and sensitive to the action of a broad spectrum of antitumor preparations belonging to the series of alkylating agents, antibiotics, antimetabolites, and alkaloids. This model is of great interest for the study of some theoretical and practical problems associated with cross resistance and ways of overcoming it.

EXPERIMENTAL METHOD

Experiments were carried out on 292 Wistar rats. Yoshida sarcoma was transplanted as ascites fluid cells in a dose of 10^6 per animal, intraperitoneally and subcutaneously. Administration of pafencyl began 72 h after transplantation. In experiments with quantitative determination of the degree of resistance, bilateral transplantation of tumors was used (sensitive on one side, resistant on the other). This method gives highly comparable results and enables the number of experimental animals to be reduced. Resistance was induced by systematic courses of pafencyl by the intragastric route in the form of a suspension in 1% starch mucilage. The antitumor activity of pafencyl was assessed by measuring the mean diameter of the tumors and also their weight after sacrifice of the animals on the 10th-14th day after administration of the drug. Statistical analysis of the data was carried out by the Litchfield–Wilcoxon method in Balen'kii's modification [1]. Transplantations continued for 32 generations, during which the rats received repeated doses of pafencyl.

EXPERIMENTAL RESULTS

In January, 1982, induction of resistance to pafencyl on Yoshida sarcoma began. The compound was given in five doses each of 1 mg/kg, after the 3rd generation in a dose of 2 mg/kg,

Research Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. K. Gavrilov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 479-481, April, 1985. Original article submitted May 29, 1984.

 0007-4888/85/9904-0514$09.50

TABLE 1. Action of Pafencyl on Ordinary and Resistant (32nd generation) Strains of Yoshida Sarcoma during Bilateral Transplantation (results and average confidence intervals at P = 0.05 level)

Dose, mg/kg	Sensitive strain			Resistant strain			Change in body weight, g
	mean weight of tumors, g	P	per cent of inhibition	mean weight of tumors, g	P	per cent of inhibition	
Control	23,6 (28,7÷18,5)	—	—	17,5 (20,4—14,6)	—	—	41,9
80	0	—	100,0	1,9 (6,21—2,41)	<0,001	91,9	—3,1
40	0	—	100,0	16,1 (19,3—12,9)	>0,1	8,0	21,1
20	0,06 (0,1—0,01)	<0,001	99,7	19,5 (24,8—12,3)	>0,1	—11,4	—2,3
10	0,06 (0,2—0,02)	<0,001	99,7	16,0 (20,3—11,7)	>0,1	8,5	31,0
5	0,16 (0,42—0,1)	<0,001	99,3	23,6 (34,4—12,8)	>0,1	—34,8	—7,5
2,5	9,1 (11,5—1,7)	<0,002	61,4	30 (43,0—17.0)	>0,1	—14,2	19,1

Legend. The animals were killed on the 13th day after transplantation.

after the 13th — 4 mg/kg, after the 17th — 5 mg/kg, and after the 28th generation 4 times in a dose of 10 mg/kg. The total dose of 40 mg/kg is the maximal tolerable therapeutic dose (MTD) and causes complete regression of the initial strain of Yoshida sarcoma (Table 1).

Resistance to pafencyl develops slowly, and doubling the dose in the course of induction led to regression of the sarcoma. Induction was therefore achieved by administration of different doses, including the maximal course dose, which did not cause regression in the preceding generation, to a small group of animals.

Staggered tests of resistance showed that it increased gradually until the 16th generation. A dose of 40 mg/kg, for instance, caused virtually complete regression of an ordinary tumor — inhibition by 99.6% ($P < 0.001$), whereas a dose of 20 mg/kg led to 99.8% inhibition ($P < 0.001$). Under these circumstances the weight of the tumor when a larger dose was used was 0.05 g, when a smaller dose was given it was also 0.05 g, and the mean weight in the control was 23 g ($P = 0.05$).

The mean weight of tumors of the 16th generation of the resistant strain was reduced from 33.3 g ($P = 0.05$) in a dose of 40 mg/kg to 9.8 g, and in a dose of 20 mg/kg to 15.3 g. The percentage of inhibition was 70.6 and 54.0 respectively ($P < 0.001$).

Subsequent systematic administration of increasing doses of pafencyl caused an increase of resistance, when MTD of pafencyl did not inhibit growth of the tumor, whereas a smaller dose actually stimulated its growth.

Further study of the action of a spectrum of doses of pafencyl on sensitive and resistant (32nd generation) strains of sarcoma during transplantation in one host revealed clear differences in their sensitivity. Table 1 shows that significant inhibition of tumor growth was obtained only in a toxic dose (LD_{50}) of 80 mg/kg (Table 1). All tolerable doses, causing complete regression of the ordinary Yoshida sarcoma did not inhibit growth of the resistant tumor or stimulated it.

Systematic repeated administration of pafencyl in increasing doses, starting from 5 and going on up to 40 mg/kg per course, thus causes a gradual increase in resistance of the cells to its action. After the 25th-30th generations of transplantations and treatment with pafencyl, their resistance remained stable.

The index of resistance to pafencyl, i.e., the ratio of the dose causing 50% inhibition of the resistant strain (32nd generation) and the dose inhibiting growth of the ordinary strain of Yoshida sarcoma by 50%, was 30.9.

The new resistant strain, virtually insensitive to the action of MTD of pafencyl, thus obtained can be used to study the mechanism of development of resistance, cross resistance to different drugs, and ways of overcoming it.

The resistant strain can be preserved in the frozen state at —196°C. The cells maintain a stable level of resistance under those conditions for over 2 years.

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STRAINS OF HUMAN MELANOMA TRANSPLANTED INTO NUDE MICE AND RATS

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UDC 616-006-089.843-06:616.438-089.87]-092.9

KEY WORDS: melanoma; strains; nude mice; nude rats.

Various tumors have now been transplanted into nude mice and rats directly from man or after passage through tissue culture. Serial passage of these tumors in nude animals in some cases leads to the formation of tumor strains with stable characteristics. The obtaining of various transplantable strains of human tumors and, in particular, sets of strains of tumors identical in histogenetic features, is important for experimental cancer research.

This paper describes a series of strains of human melanoma in the collection of the All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR.

EXPERIMENTAL METHOD

Nude mice based on line BALB/c, 6-8 weeks old, and nude rats 4-6 weeks old, of our own breeding, were used. Human tumors obtained from operation material were transplanted subcutaneously into mice in the form of fragments. In one case human melanoma cell line MeWo was used for transplantation [4]. In this case 10^7 cells in 0.5 ml were injected subcutaneously into mice. A suspension containing 150 mg of human tumors, previously subjected to serial passage in mice, in 0.5 ml was injected subcutaneously into nude rats. In every case, both in nude mice and in nude rats after the second passage of the tumor, the suspension was transplanted serially and subcutaneously. The species to which tumors growing in nude animals belonged was determined by electrophoresis of lactate dehydrogenase in agar gel. Histological characteristics of the tumors were studied by the use of sections stained with hematoxylin and eosin, with picrofuchsine, and by the PAS reaction.

For electron-microscopic investigation the tumor tissue was fixed in a 2.5% solution of glutaraldehyde, postfixed in OsO_4 solution, dehydrated in alcohols of increasing concentration, and embedded in Epon 812 resin mixture. Semithin and ultrathin sections were cut on LKB-111 ultramicrotomes and the ultrathin sections were examined in the JEOL-100C electron microscope. Semithin sections were stained with toluidine blue, ultrathin with an aqueous solution of uranyl acetate and lead citrate.

EXPERIMENTAL RESULTS

Four strains of human melanoma transplantable into nude mice and rats were obtained; three strains (Mel-2, Mel-3, and Mel-5) were obtained from operation material.

Strain Mel-2, transplanted into nude mice and rats with an interval of 27-30 days, has undergone 53 passages. The tumor which was the source for this strain corresponded in structure to the typical picture of an alveolar-solid epithelial-like melanoma, producing pigment (Fig. 1a). Strain Mel-2 had an alvolar-lobular structure and consisted of large cells with

Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 481-483, April, 1985. Original article submitted August 24, 1984.

 0007-4888/85/9904-0516$09.50